American Journal of Plant Physiology 3 (2): 50-66, 2008
ISSN 1557-4539

Recognition, Implication and Management of Plant Resistance to Herbicides

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Abstract: Herbicides control undesired grasses and weeds while leaving the crop relatively unharmed. Herbicide antidotes (safeners) protect crops from herbicide damage without protecting weeds. Resistance to herbicides means the survival of a segment of the population following treatment with an herbicide dosage lethal to the normal population. Three mechanisms that account for herbicide resistance are: alterations in the target site, enhanced metabolism and compartmentation of the herbicide. Plants metabolize most herbicides through a series of intermediates ultimately to non toxic compounds. The basic detoxification reactions are oxidation, reduction, hydrolysis and conjugation. Major oxidative reactions are catalyzed by Cytochrome P450 monooxygenases (P450s) while the enzymatic conjugation with glutathione (GSH) catalyzed by glutathione S-transferases (GSTs) is the major detoxification pathway. Resistance occurs in plants as the result of random and infrequent mutations. Cross-resistance refers to resistance to an herbicide the plant has not been previously exposed to but that has a mode of action similar to the original herbicide. Multiple-resistance refers to resistance to more than one class of herbicides with very different modes of action. Most of uses of molecular biology are to find new herbicide targets and to generate herbicide-resistant crops by inserting exogenous resistance genes into the crops or by selecting natural mutations. Unlike the generation of transgenics, recurrent selection of a natural mutant will select for homozygous resistant individuals. Transgenics bear and express both the native and transgenic enzymes; they are functionally heterozygous and will remain so. Nevertheless, precautions must be considered for risk assessment. The main risk is claimed to be that of the biotechnologically-derived herbicide resistant crops becoming 'volunteer' weeds, or their introgressing traits into a wild relatives. The final decision on risk/benefit is ultimately a balance between science, economics, local benefits, local values, pressure groups and local politics.

Key words: Herbicide resistance, herbicide safeners, cross resistance, resistance management

INTRODUCTION

Herbicide activity is dependent on the efficiency of absorption, translocation, metabolism and biochemical action (Preston, 2004; Wang *et al*., 2007). The selectivity of a compound between target and non-target organisms may depend on complex interspecific differences in the efficiency of these processes. However, minor differences in chemical structure may lead to considerable differences in selectivity. According to their chemical structure, members of each herbicidal class would have most likely similar mode of action, may tend to induce more or less similar physiological characteristics.

Resistance to herbicides means the survival of a segment of the population following treatment with an herbicide dosage lethal to the normal population (Holtum *et al*., 1994; Powles and

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Holtum, 1994). Herbicide resistance is the inherited ability of a weed population to survive a herbicide application that is normally lethal to the vast majority of individuals of that species (Powles *et al*., 1998). Weed populations evolve herbicide resistance through selection pressure imposed by frequent use of one or more herbicides with the same mode of action or metabolic degradation pathway at a location over an extended period of time (Valverde *et al*., 2000). Pedersen *et al*. (2007) stated that repeated use of glyphosate has resulted in evolution of glyphosate-resistant *Lolium rigidum* populations in Australia. They indicated important differences in resource allocation during the reproductive stage for resistant and susceptible phenotypes. However, tolerance to herbicides means the survival of normal population of a species following treatment with an herbicide dosage lethal to other species. Tolerant biotypes are only partially affected by the herbicide (Devine and Preston, 2000). The different levels of tolerance of crops and weeds to specific herbicides are the basis of selectivity. Two types of genes have been used to generate herbicide-resistant crops; where the gene product detoxifies the herbicide and where the herbicide target has been modified such that it no longer binds the herbicide. On the other hand, Sandermann (2006) reported that a single weed population was found to contain five biotypes whose response to glyphosate ranged from sensitive to four times more tolerant than the sensitive biotype. These biotypes had developed in the absence of glyphosate while remaining sensitive to two other herbicides. Multiple biochemical resistance mechanisms and underlying genes were demonstrated. Moreover, Gui-su *et al*. (2007) indicated that the heterozygous populations and isogenic lines with homocaryotic alloplasmic genes were obtained by crossing and reciprocal crossing of cytoplasmic herbicide resistant plants with susceptive plants of foxtail millet. A fitness penalty with any target site herbicide resistant crops could be assumed. Some biotypes can have a seemingly absolute resistance; they can be treated with saturated solutions of herbicides that have little or no effect on the plant. Some resistant biotypes are controlled by concentrations of herbicides but at application rates many times greater than the recommended dose of the herbicide. So, this article was aimed to overview plant resistance to herbicides; the main prospects, implication, recognition and management to declare the risk/benefit balance for endowing herbicide-resistant species.

Herbicide Safeners

Safeners are compounds that are utilized to protect crops from herbicidal injury without protecting weeds. They are chemically diverse compounds with the ability to selectively protect crop species from herbicides. The development of antidotes permits the use of certain herbicides on crops, which would normally be affected by the herbicide such as naphthalic anhydride, benoxacor, dichlormid cyometrinil, flurazol and oxabetrinil. However, interference with herbicide uptake and translocation, reduced sensitivity of the target site and enhanced herbicide degradation are among the mode of action of herbicide antidotes (Davis and Caseley, 1999). Nemat Alla and Hassan (1998) reported that naphthalic anhydride and flurazol protected maize from metolachlor toxicity mainly by stimulating GSH accumulation and GST activity. Fonne-Pfister *et al*. (1990) found that the protection from sulfonylurea phytotoxicity by naphthalic anhydride resulted from an increased rate of sulfonylurea herbicides metabolism by hydroxylation and glucose conjugation. Treatment of sorghum seeds with flurazol, naphthalic anhydride, benoxacor or dichlormid protected seedlings from growth inhibition by alachlor (Hirase and Molin, 2001). They further indicated that all of these safeners increased the activity of cysteine synthase. Similarly, Nemat Alla (2000) found that naphthalic anhydride relieved the toxicity of alachlor, metolachlor and atrazine in maize through induction of GST isoforms and GSH contents.

Genetic Nature of Resistance

Resistance may occur in plants naturally due to selection as the result of random and infrequent mutations or it may be induced through genetic engineering (Gressel, 2000; Devine and Preston, 2000).

Through selection, where the herbicide is the selection pressure, susceptible plants are killed while resistant plants survive to reproduce without competition from susceptible plants. If the herbicide is continually used, resistant plants successfully reproduce and become dominant in the population (Pedersen *et al*., 2007). In the absence of herbicide treatment, resistant species to an herbicide are not as fit as are susceptible plants. This is because the efficiency of some physiological processes such as photosynthesis is reduced in resistant plants by the alteration of a specific protein that is also the herbicide binding site, so conferring resistance.

Since resistant plants are less fit, they reproduce at lower rates and consequently represent a smaller fraction of the number of individuals within a population. In contrast, some resistance traits do not have the same fitness cost. In those cases, resistant individuals often represent a larger fraction of a population. Consequently, increased tolerance to most herbicides is generally inherited in a polygenic fashion; more than one allele on many genes gives additive tolerance. Some increases in tolerance are due to gene duplications (Gressel and Rotteveel, 2000). This could give rise to higher levels of detoxifying enzymes. Resistance is usually inherited on one or at most two major nuclear genes in species where newly resistant biotypes or varieties have appeared (Cole and Rodgers, 2000; Devine and Preston, 2000; Gressel, 2000). Therefore, herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide that would normally be lethal to the wild type.

The mutation theory postulates that a genetic mutation occurs within a plant following the application of an herbicide and that this mutation confers resistance to the plant (Daniell, 1999). Repeated use of herbicides that target the same site of action, or are broken down in plants through similar biochemical pathways, can lead to the selection of individuals with a genetic endowment to survive lethal doses of the herbicide. On the other hand, Jander *et al*. (2003) reported that the spontaneous mutations giving rise to glyphosate resistance occur at low frequencies and may be associated with pleiotropic fitness costs. Different mutations on the target ALS enzyme can lower its affinity for a wide range of ALS inhibitors, resulting in various patterns of resistance to these herbicides. However, Fischer *et al*. (2000) have demonstrated that resistance to bispyribac-sodium in an *Echinochloa phyllopogon* biotype did not involve an altered target site, but instead, a mechanism of enhanced degradation was possibly mediated by P450. In accordance, tolerance of rice to bispyribac-sodium can be abolished by the simultaneous application of P450 inhibitors suggesting the involvement of P450 as a mechanism of selectivity to this herbicide in rice (Osuna *et al*., 2002).

The natural selection theory is widely regarded as the most plausible explanation for the development of resistance. The theory states that herbicide-resistant species have always occurred at extremely low numbers. Most herbicides affect a single specific site of action and that site is usually under the control of a single gene, or at most a few genes (Gressel, 2000). With a single gene mutation, even minor changes in gene expression can confer resistance by modifying the site where an herbicide has its toxic effect; the site of action. The evolution of a resistant population in a species comes about in response to selection pressure imposed by that herbicide or by another herbicide that shares the same site of action. When a herbicide exerts selection pressure on a population, plants possessing the resistance trait have a distinct advantage (Daniell, 1999). When an herbicide effectively controls the majority of susceptible members of a species, only those possessing a resistance trait can survive and produce seeds for future generations. As known, plants exhibit a wide range of diversity. The plants in a population with characteristics enabling them to survive under a wide range of environmental and other adverse conditions will be the ones to produce seeds that maintain these survival characteristics. The plants less adapted do not survive and hence only the fittest plants produce seeds. Plants that possess characteristics, such as resistance to herbicides that are not common to the entire species are referred to as biotypes. When most of the susceptible members of a population are controlled, those resistant biotypes are able to continue growing and eventually to produce seeds. The seed from the

resistant biotypes ensures that the resistance trait carries into future seasons. If the same herbicide is used year after year, or several times during a single season, the resistant biotypes continue to thrive, eventually out-numbering the normal (susceptible) population (Powles *et al*., 1998). Consequently, relying on the same herbicide (or herbicides with the same mode of action) for weed control creates selection pressure that favors the development of herbicide-resistant biotypes.

Contributing Factors for Plant Resistance

Three mechanisms have been identified that account for herbicide resistance (Devine and Preston, 2000). The first deals with alterations in the target site of the herbicide. An herbicide has a specific site within the plant where it acts to disrupt a particular plant process or function. If this target site is somewhat altered, the herbicide molecule may be unable to exert its phytotoxic action effectively. Thus, most cases of herbicide resistance have involved alterations in the herbicide target site such as photosystem II (PSII), acetolactate synthase (ALS), 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) and acetyl CoA carboxylase (ACCase). Target site insensitivity is the most commonly reported mechanism of herbicide resistance. Herbicide resistance caused by target site changes often provides high levels of resistance (Preston, 2004) and is often dominant. Dinelli *et al*. (2005) concluded that there are three factors may concur to glyphosate resistance in the investigated resistant biotypes: impaired translocation of the herbicide, increase in EPSPS transcript levels and enhanced ramification. They further indicated differences in glyphosate uptake, translocation, or metabolism were disregarded as potential resistance mechanisms in *L. rigidum*, suggesting that resistance may be conferred by EPSPS overexpression, an insensitive EPSPS, or improper targeting of glyphosate to the loci of action (Lorraine-Colwill *et al*., 1999). Further, the mechanism of resistance in *L. rigidum* was credited to differences in cellular translocation of glyphosate (Lorraine-Colwill *et al*., 2003).

The second mechanism is the enhanced metabolism of the herbicide. Metabolism within the plant is one mechanism a plant uses to detoxify foreign compounds such as herbicides. A plant with an enhanced ability to metabolize an herbicide can potentially inactivate it before it can reach its site of action within the plant. Compartmentation of the herbicide represents the third mechanisms for herbicide resistance. Plants are capable of sequestering foreign compounds within their cells or tissues to prevent the compounds from causing harmful effects. When an herbicide is placed within a restricted compartment, it cannot reach its site of action and thus is unable to kill the plant. The proposed mechanism for this resistance is that the resistant biotypes restrict the movement of the herbicides within themselves and do not allow the herbicides to reach their sites of action. A high rate of seed production with most seed germinating within a year can accelerate the evolution of resistance. When susceptible plants are removed from the population by the herbicide, prolific seed production by resistant plants rapidly shifts the population toward resistance. High seed production coupled with genetic variation increases the probability that resistance will evolve. Herbicides with prolonged soil residual activity exert selection pressure for a longer time period since they kill most of the susceptible plants that germinate over a growing season. An herbicide with a single target site controlled by few genes is more likely to encounter plants with mutations for resistance than is an herbicide with several modes of action (Gronwald, 1994). A high effective kill rate rapidly depletes susceptible genes from the population and the result is a rapid increase in resistance among the progeny of a few initial resistant plants (Orson and Oldfield, 1999).

Like target site changes, selection for enhanced metabolism can also occur in response to repeated applications of the same herbicide or of a group of herbicides that are vulnerable to the same detoxification enzymes. Selection with enhanced metabolism is more rapid when an herbicide is used continuously at or below the low recommended rate. This allows a gradual increase of the biotypes that are more able to metabolize the compound. It is well established that persistent herbicide application to a plant population is a strong selection pressure for individuals carrying genes conferring herbicide

resistance. Biotypes with enhanced metabolism have a lower level of resistance than those expressing resistance through site of action changes. Plants expressing any genetically-endowed traits enabling survival in the presence of the herbicide have a strong advantage and may come to dominate the population. The severity and time-period over which resistance can develop varies dependent upon the herbicide(s) used and biological, agro-ecological and managerial factors (Powles and Holtum, 1994).

Cross Resistance to Herbicides

Cross resistance is defined as the expression of a genetically-endowed mechanism conferring the ability to withstand herbicides from different chemical classes. There are two broad cross resistance categories; target site cross resistance and non target site cross resistance (Hall *et al*., 1994).

Target Site Cross Resistance

It occurs when a change at the biochemical site of action of one herbicide also confers resistance to herbicides from different chemical classes that inhibit the same site of action in the plant. Target site cross resistance does not necessarily result in resistance to all herbicide classes with a similar mode of action or indeed all herbicides within a given herbicide class. Herbicides are active at one or more target sites within a plant. Target sites are enzymes, proteins, or other places in the plant where herbicides bind and thereby disrupt normal plant functions (Saari *et al*., 1994). One example is ALS that is involved in synthesizing branched-chain amino acids, valine, leucine and isoleucine. Several classes of herbicides are known to inhibit ALS, such as the sulfonylureas, imidazolinones, triazolopyrimidine sulfonanilides and pyrimidinyl oxybenzoates, by binding to a relic quinone-binding site (Tan and Medd, 2002), causing dysfunction of the enzyme and reducing the synthesis of certain amino acids that are necessary for protein synthesis (Nemat Alla and Hassan, 1998; Devine and Preston, 2000). These highly selective ALS-inhibiting herbicides are very valuable for weed management in a wide range of crops worldwide. These ALS-inhibiting herbicides differ in chemical structure but are active at the same target site. Plants resistant to ALS herbicides have altered ALS that does not bind the herbicide. Often, a resistant species that has been selected by pressure from one herbicide will be resistant to all herbicides that act on that herbicide's target site (Hall *et al*., 1994). When a plant expressing resistance to an herbicide also demonstrates resistance to other herbicides that target the same plant process even though the plant has not been exposed to the other herbicides, the resistance is termed cross-resistance on a target-site basis (target-site cross-resistance). Consequently, cross-resistance refers to resistance to an herbicide the plant has not been previously exposed to but that has a mode of action similar to the original herbicide.

E. phyllopogon has evolved resistance to several herbicides, including bispyribac-sodium (ALS inhibitor), which has not yet been commercially used (Osuna *et al*., 2002). They detected the involvement of P450s in *E. phyllopogon* resistance to bensulfuron-methyl using the P450 inhibitors piperonyl butoxide and malathion. The dose-response studies confirmed cross-resistance in resistant *E. phyllopogon*. ALS assays demonstrated that, unlike resistant *E. phyllopogon*, cross-resistance in resistant *Cyperus difformis* was due to reduced ALS sensitivity. Thus, binding differences between both herbicides at the target site are suggested. The study of Osuna *et al*. (2002) reveals that cross-resistance between bensulfuron-methyl and bispyribac-sodium in both weeds involves degradation enhancement through monooxygenases and target site alteration.

The considerable variation in the level of resistance across and within various ALS-inhibiting herbicide chemistries is likely to be due to subtly different binding by particular herbicides on the ALS enzyme and different mutations of ALS. Evidences from competitive binding studies show that ALS-inhibiting herbicides bind to the same or closely overlapping sites on ALS (Devine and Preston, 2000). The wide variation in target site cross resistance amongst biotypes with resistant ALS enzyme implies that there are a number of different functional mutations of the ALS gene. Resistant biotypes in many cases have modified ALS genes with one or more point mutations causing reduced sensitivity to the ALS-inhibiting herbicides (Tan and Medd, 2002). Substitution in any of the five

conserved amino acids (Ala_{122}, Pro_{197}, Ala_{205}, Trp_{574}, or Ser_{653}: numbered based on the ALS precursor in *Arabidopsis thaliana*) is known to result in ALS inhibitor resistance. In addition, Holtum *et al.* (1994) stated that ALS gene sequences from a number of resistant biotypes of higher plants show a substitution at a proline residue (173) in a highly conserved region of the enzyme, known as domain A. Substitutions of threonine, alanine, serine, histidine and glutamine for this proline residue have been observed in some species. There are several possible mutations of the ALS gene, which will confer resistance to these herbicides and yet retain enzyme function. It is likely that these different mutations in the ALS gene provide different levels of target site cross resistance within and between ALS-inhibiting herbicide chemistries.

A *Rotala indica* accession was tested for resistance to the sulfonylurea herbicide, imazosulfuron (Kuk *et al.*, 2002). The accession was confirmed to be resistant and was cross-resistant to other sulfonylurea herbicides, bensulfuron-methyl, cyclosulfamuron and pyrazosulfuron-ethyl, but not to imidazolinone herbicides, imazapyr and imazaquin. The resistance mechanism of *R. indica* to imazosulfuron was mainly due to an alteration in the target enzyme, ALS. Since the level of resistance to other sulfonylurea herbicides in the enzyme assay was much lower than that in the whole plant assay, other mechanisms of resistance, such as herbicide metabolism, or reduced absorption and translocation may be involved. Many researchers have shown that the mechanism of resistance to ALS inhibitors is alteration of the target enzyme (Saari *et al.*, 1994; Hwang *et al.*, 2001). A number of different mutations can endow resistance to various ALS-inhibiting herbicides without any significant impairment of enzyme function *in vivo*. This is also likely to be the case for herbicide-resistant ACCase (Heap, 2000) and EPSPS (Pedersen *et al.*, 2007), but is not the case for herbicide-resistant PSII (Mengistu *et al.*, 2000) in which very few mutations confer resistance and yet retain full enzyme functionality. Two chemically dissimilar herbicide groups, the aryloxyphenoxypropionic acid and cyclohexanedione herbicides target the enzyme ACCase (Devine and Shimabukuro, 1994). These herbicides are lethal to many Graminaceae but are harmless to dicot species and have therefore become widely employed for grass weed control.

Varying levels of resistance to haloxyfop-R-methyl and sethoxydim, the Accase inhibitors, were found in itchgrass (*Rottboellia cochinchinensis*) biotypes and cross-resistance among graminicides was confirmed (Avila *et al.*, 2007). No differences in the translocation or metabolism of sethoxydim were observed between resistant and susceptible biotypes. In *in vitro* ACCase assays, the concentrations of sethoxydim required to inhibit ACCase activity by 50% were substantially higher (about 11 times) for the resistant biotypes compared to the reference biotype, indicating that the resistant itchgrass biotypes have an ACCase that is relatively insensitive to the graminicides. These results suggest that cross resistance in itchgrass biotypes is conferred by a reduced sensitivity of the target enzyme. Selection either with an aryloxyphenoxypropionic acid herbicide, or a cyclohexanedione herbicide, has led to target site cross resistance to both classes. Two biotypes of *Alopecurus myosuroides* have been documented as highly resistant to the aryloxyphenoxypropionic herbicides as a result of resistant ACCase (Hall *et al.*, 1994). The ACCase from these biotypes is also resistant to cyclohexanedione herbicides. Resistance to aryloxyphenoxypropionic acid herbicides in several biotypes of the wild oat species *Avena fatua* and *Avena sterilis* is also endowed by resistant forms of the ACCase enzyme (Devine and Preston, 2000). Herbicides from different chemical classes bind to overlapping, but not identical sites on the target enzyme. The patterns of resistance of ACCase to herbicides can be strikingly different even among resistant biotypes of the same species. Moreover, aryloxyphenoxypropionic acid and cyclohexanedione herbicides cause also a rapid depolarization of plant cell membrane potentials by allowing the influx of protons (Shimabukuro, 1990). This ability to depolarize the membrane potential following removal of the herbicide is not observed with susceptible biotypes. Powles and Holtum (1994) recorded the occurrence of repolarisation of the membrane potential in resistant *L. rigidum* biotypes irrespective of the possession or absence of a

resistant ACCase. DiTomaso (1993) claimed a direct connection between the differential abilities of the resistant *L. rigidum* biotypes to acidify the external medium and the repolarisation of the membrane potential following removal of the herbicide.

Changes to the EPSPS target enzyme was reported following glyphosate application. However, the changes of the enzyme appear to be less frequent than changes in glyphosate translocation processes (Duke, 2005; Koger *et al*., 2005). Multiple biochemical factors appear to contribute to resistance and an incompletely dominant single locus nuclear gene has been linked to glyphosate resistance (Zelaya *et al*., 2004; Owen and Zelaya, 2005). Biochemical studies point to reduced translocation of glyphosate as a major reason for resistance (Vaughn, 2003; Feng *et al*., 2004; Koger and Reddy, 2005). Resistance to glyphosate has been reported in *Eleusine indica* (Lee and Ngim, 2000) and in *Conyza canadensis* (Main *et al*., 2004). The primary mode of action of glyphosate is the competitive inhibition of the plant enzyme EPSPS which catalyses the penultimate step in the shikimate pathway (Franz *et al*., 1997). Studies of the molecular and genetic basis of evolved resistance to glyphosate in biotypes of *E. indica* have identified target-site-based resistance due to single nucleotide substitutions within the EPSPS gene endowing an insensitive EPSPS enzyme (Baerson *et al*., 2002; Ng *et al*., 2003). Feng *et al*. (2004) found that glyphosate resistance in populations of *C. canadensis* was also strongly correlated with impaired translocation of glyphosate.

Another target site is the photosynthetic electron transfer at Photosystem-II (PSII), which is inhibited by numerous chemically dissimilar herbicide classes such as triazines and substituted ureas (Mengistu *et al*., 2000). PSII is an essential component of the photosynthetic apparatus in plants that uses light energy to split water, releasing oxygen, protons and electrons. The electron flow from PSII is essential to plant life. Mature D1 protein is an integral part of PSII. The D1 protein has an extraordinarily high rate of turnover, faster than any other known thylakoid protein. Continual synthesis of D1 protein is necessary to replace photo-damaged D1, which is then quickly degraded by proteolysis (Rintamaki *et al*., 1996). Classical PSII inhibitors, such as the herbicides triazines and phenylureas, bind to the D1 protein in a stoichiometric fashion. Upon herbicide binding, the electron flow from PSII is disrupted and carbon dioxide fixation ceases. Since the electron acceptor in the inhibited PSII is now not able to accept electrons from photo-excited chlorophyll, free radicals are generated and chlorosis develops (Rutherford and Krieger-Liszkay, 2001). These herbicides bind to the binding site on the D1 protein within the PSII reaction center. Herbicide resistance is due to target site resistance endowed by a modification at the herbicide target site, the D1 protein of PSII (Gronwald, 1994). It is noteworthy that biotypes highly resistant to triazine herbicides as a result of a modified D1 protein are not resistant to the chemically distinct substituted urea herbicides, despite the fact that the substituted urea herbicides are also potent PSII inhibitors (Gronwald, 1994). A plausible explanation is that the substituted urea and triazine herbicides probably bind to overlapping, but not identical, sites in PSII (Trebst, 1991).

Non Target Site Cross Resistance

It is defined as cross resistance to dissimilar herbicide classes conferred by a mechanism(s) other than resistant enzyme target sites. Heap and Knight (1986) elucidated that many *L. rigidum* populations that developed resistance following selection with the ACCase-inhibiting herbicide diclofop-methyl display resistance to ALS herbicides without any exposure to ALS herbicides; this means non target site cross resistance. Similarly, Matthews (1994) showed that an initially susceptible *L. rigidum* population when selected for three generations with diclofop-methyl developed resistance to diclofop-methyl and simultaneously exhibited resistance to the ALS-inhibiting herbicide chlorsulfuron without any exposure to chlorsulfuron. Thus selection with an ACCase-inhibiting herbicide can lead to resistant populations that display non target site cross resistance to ALS-inhibiting herbicides without exposure to these herbicides. Therefore, cross resistance to ALS

herbicides from selection with ACCase herbicides is not due to resistance at the ALS target enzyme. The resistance of these biotypes is probably resulted from an enhanced rate of herbicide metabolism by P450s. Sweetser *et al.* (1992) indicated that wheat is resistant to many ALS-inhibiting herbicides as a result of rapid metabolism of these herbicides by aryl-hydroxylation, catalyzed by a P450. Malathion which inhibits the P450-dependent detoxification of primisulfuron, a sulfonylurea herbicide, in microsome preparations from maize can inhibit chlorsulfuron metabolism and reduce chlorsulfuron resistance in the cross-resistant biotype (Christopher *et al.*, 1994). This reversal of resistance by malathion confirms that detoxification plays a major role in chlorsulfuron resistance in this biotype. On the other hand, the rate of metabolism of diclofop-acid in a resistant *L. rigidum* biotype occurs at about 1.5 times the rate observed in a susceptible one (Holtum *et al.*, 1991). A considerable proportion of the diclofop acid, about 20 % in resistant and 30 % in susceptible biotypes remains unmetabolised even 192 h after treatment (Holtum *et al.*, 1994). The location of this remaining herbicide might be sequestered away from the metabolizing enzymes and the active site. It appears likely that enhanced metabolism is the common mechanism of herbicide resistance operating in the resistant biotype.

Similarly, a *L. rigidum* biotype selected with a substituted urea herbicide (diuron) displays resistance to diuron, which also extends to a range of other urea herbicides and exhibits cross resistance to triazine herbicides (Burnet *et al.*, 1993a). The mechanism endowing triazine and substituted urea herbicide resistance has been identified in these biotypes as due to enhanced rates of herbicide metabolism (Burnet *et al.*, 1993a, b). The triazine herbicide simazine is metabolized in the resistant biotypes 2 to 3 times the rate of metabolism attained by the susceptible biotype (Burnet *et al.*, 1993b). Similarly, the substituted urea herbicide chlortoluron is also metabolized at an enhanced rate (Burnet *et al.*, 1993a). The developed resistance of *Alopecurus myosuroides* biotypes following selection with substituted urea herbicides, particularly chlortoluron is not due to a resistant PSII target site but due to an enhanced rate of metabolism of chlortoluron and isoproturon (Hall *et al.*, 1994), which is probably endowed by increased activity of P450 catalyzed metabolism. 1-aminobenzotriazole, piperonyl butoxide and tetcyclasis, three potent, broad-spectrum inhibitors of P450 enzymes, inhibit metabolism of chlortoluron and antagonize resistance (Burnet *et al.*, 1993a). Similarly 1-aminobenzotriazole inhibits simazine metabolism and reduces the level of simazine resistance (Burnet *et al.*, 1993b). 1-aminobenzotriazole inhibited metabolism of chlortoluron and isoproturon. Microsomal membrane preparations isolated from a resistant and a susceptible population displayed low intrinsic rates of chlortoluron metabolism (Holtum *et al.*, 1994). Nemat Alla (2000) found that 1-aminobenzotriazole increased phytotoxicity of alachlor, metolachlor and atrazine concluding greater persistence in contact with their target sites. Moreover, greater persistence of these herbicides was concluded as a result of P450 blockage, which would retract their degradation. Therefore, the enzymic basis for the enhanced metabolism resistance mechanism in these biotypes is likely to be due to increased activity of P450s, which have the capacity to either de-alkylate or ring-hydroxylate these herbicides.

Multiple Resistance to Herbicides

Multiple resistance is the expression-within individuals or populations- of more than one resistance mechanism. Multiple resistant plants may possess from two to many distinct resistance mechanisms and may exhibit resistance to a few or many herbicides. When a plant that has been exposed to herbicides that attack different target sites expresses resistance to more than one of these herbicides, that is termed multiple resistance (Hall *et al.*, 1994). The simplest cases are where an individual plant (or population) possesses two or more different resistance mechanisms which provide resistance to a single herbicide, or class of herbicides. More complicated are situations where two or more distinct resistance mechanisms have been selected either sequentially or concurrently by different herbicides and endow resistance to the classes of herbicide to which they had been exposed. The most complicated and difficult to control situations are where a number of resistance mechanisms, involving

both target site and non target site resistance mechanisms, are present within the same individual (Powles and Holtum, 1994). Most cases of herbicide resistance in plants involve a single mutation or modification in some function so that the species is resistant or cross-resistant. Rarely does a single plant express resistance to several herbicides that affect different target sites. The mechanisms of multiple resistance in the plant include changes to the herbicides' sites of action and the detoxification of herbicides by P450s (Barrett, 2000). Consequently, multiple-resistance refers to resistance to more than one class of herbicides with very different modes of action in which more than one basis for resistance is involved. Multiple resistance has been reported on a number of occasions when herbicides of different chemical classes have been applied to a population either as a mixture, or sequentially, following the development of resistance to the first herbicide (Hall *et al*., 1994; Barrett, 2000). A biotype of *Amaranthus retroflexus* was developed target site resistance to triazine herbicides and when diuron was applied to this resistant population, resistance developed to diuron (Lehoczki *et al*., 1991).

Molecular Biology for Herbicide Resistance

The emerging field of molecular ecology aims to improve the ecological predictability of transgenic crop plants (Sandermann, 2006). Selectivity between crop and weed are due to catabolic degradation of the herbicide by the crop, closely related weeds are to be expected to have similar catabolic pathways as the crop. Herbicides can interfere with many different plant processes, usually acting at a single molecular site, generating profound metabolic consequences that lead eventually to the death of the plant. Herbicide resistance can be conferred by several mechanisms, the most important of which are target site insensitivity and rapid metabolic transformation of the herbicide to inactive products (Devine and Preston, 2000). The ability to transform any major crop with herbicide tolerance genes means that new uses for individual herbicide chemicals can be created, maximizing selectivity between weeds and the crop by manipulating the properties of the crop plant, rather than chemistry. Selectivity is enhanced by inserting exogenous resistance genes into the crops or by selecting natural mutations.

Biotechnologically-Derived Herbicide Resistant Crops

The real values of biotechnologically-derived herbicide resistant crops come from instances where there really are no viable weed control methods (e.g. due to evolved herbicide resistances in weeds) (Cole and Rodgers, 2000). The easiest way to obtain selectivity among closely related species is to engineer resistance to a general herbicide into the crop (Sankula *et al*., 1997). The calibrational use of antisense and knockout techniques with known herbicide targets yields inhibited or dead plants (Haake *et al*., 1998). So, biotechnology has been used to generate target site herbicide resistant crops by transfer of field or laboratory generated mutants into crop varieties. As all the mutations were found at a low frequency; one in a million or less, the resistant traits are not of near neutral fitness. Most ALS mutant enzymes are insensitive only to certain classes of ALS inhibitors (Guttieri *et al*., 1995). The *csr1-1* gene encodes an enzyme only insensitive to sulfonylureas whereas the imr1 mutant ALS gene also isolated from *Arabidopsis thaliana* is insensitive to imidazolinones (Sathasivan *et al*., 1991). By creating suitable hybrid ALS gene from both of these mutants, transgenic tobacco biotypes could be generated displaying high level resistance to both classes of herbicides. On the other hand, EPSPS is a single polypeptide in plants and microorganisms. It is a key pathway linking photosynthetic carbon reduction to the synthesis of aromatic amino acids, auxin and diverse secondary products in plants. Therefore, glyphosate which inhibits EPSPS results in gross depletion of aromatic amino acids (Cole and Rodgers, 2000). A fitness penalty with any target site herbicide resistant crops could be assumed due to the fact that if the resistant mutation was neutral there would be naturally-resistant populations pre-existing. Also, glufosinate resistance was similarly achieved in tissue culture by over-expression of the gene encoding its target site with overproduction of glutamine synthase (Sankula *et al*., 1997, 1998). Additionally, transgenic tobacco plants overexpressing protoporphyrinogen IX oxidase (protox) five-fold in chloroplasts are resistant to a discriminatory dose of 20 mM acifluorfen, which severely inhibited the wild type (Lermontova and Grimm, 2000).

The results of incomplete suppression of gene expression achieved by antisense or overexpressive co-suppression have an advantage over the knockout or deletion of genes for elucidating potential targets. Biotechnologically-derived herbicide resistant wheat and rice are obtained from the insertion of a gene into wheat or rice conferring resistance to a broad spectrum herbicide (Gressel, 1999a, b). The transgenes will allow problems of resistance to be overcome. Two types of gene have been used to generate herbicide-resistant crops; where the gene product detoxifies the herbicide and where the herbicide target has been modified such that it no longer binds the herbicide. Nevertheless, there have been more problems with the transgenics than the mutants. The magnitude of resistance with transgenics is not as high as it is with the natural mutations (Gruys *et al*., 1999). Transgenics will be less resistant than target site mutants bearing the same transversion. Recurrent selection of a natural mutant will select for homozygous resistant individuals. This cannot happen with the generation of transgenics, which bear and express both the native and transgenic enzymes. Thus, transgenic plants with target site resistance are functionally heterozygous and will remain so despite recurrent selection. When the herbicide is applied, target site of biotechnologically-derived herbicide resistant crops must depend on the transgenic derived enzyme while the native is inhibited; perhaps even causing phytotoxic precursors to accumulate (Lee *et al*., 2000). So, problems with some resistant crops still found, suggesting that the critical balance of transgenic and native enzymes is not optimal. Andrew (2000) detected stem brittleness and cracking in untreated glyphosate-resistant soybean suggesting an overproduction of product leading to increased lignin formation when both the native and transgene derived enzymes are operative. The problems arising from the functional heterozygote status of transgenic target site resistant plants could be overcome by enhancing the metabolism of the herbicide through gene coding for an enzyme degrading herbicide together with target-site resistance (Mannerlof *et al*., 1997).

Metabolically-Resistant Biotechnologically-Derived Herbicide Resistant Crops

More genes for catabolic resistance to several herbicides could be able to rapidly generate herbicide-resistant crops with metabolic resistance. In addition to genes encoding insensitive target sites, some detoxification genes were also identified. Many crops bearing transgenes coding for highly specific enzymes that metabolically catabolize herbicides have been generated (Cole and Rodgers, 2000). The expression of plant P450 transgenes conferred phenylurea resistance (Inui *et al*., 2001). On the other hand, transgenes encoding maize GSTs increased the level of herbicide resistance in many plant species (Jepson *et al*., 1997). Unlike the target site resistances, the crops generated with metabolic resistances seem to be problem-free, with little metabolic load conferred by generating the small amount of enzyme needed.

Inhibitors of protox induce photodynamic death of plants within 4-6 h in bright sunlight. A gene encoding the plastid-located protox of *Arabidopsis* has been introduced into the genome of tobacco (*Nicotiana tabacum*) plants (Lermontova and Grimm, 2000; Cobb and Kirkwood, 2000). The transformants were screened for low protoporphyrin IX accumulation upon treatment with the diphenyl ether-type herbicide acifluorfen. Leaf disc incubation and foliar spraying with acifluorfen indicated the lower susceptibility of the transformants against the herbicide. The resistance to acifluorfen is conferred by overexpression of the plastidic isoform of protox. The overproduction of protox neutralizes the herbicidal action, prevents the accumulation of the substrate protoporphyrinogen IX and consequently abolishes the light-dependent phytotoxicity of acifluorfen. Similarly, strains of *Conyza bonariensis* contain a complex of enzymes capable of detoxifying the reactive oxygen species generated by the PSI blocker paraquat and keeping the plants alive until the paraquat is dissipated (Ye *et al*., 2000). Nonetheless, like the native gene, the modified gene conferred a high level of resistance to the herbicide oxyfluorfen in a seedling growth test (Yang *et al*., 2006). Jung *et al*. (2004) described the development of transgenic rice plants that overexpress a heterologous Protox gene from the bacterium *Myxococcus xanthus* (Mx). When this gene is expressed in transgenic rice plants, the Mx Protox protein is dually targeted into plastids and mitochondria via

ambiguous transit signals, increasing resistance to oxyfluorfen. Overexpression of the Mx Protox transgene in rice confers a 200-fold level resistance to oxyfluorfen over the wild-type rice (Ha *et al*., 2004, 2003; Jung *et al*., 2004). Higher herbicide resistance in transgenic plants expressing Mx Protox is simply due to the overexpression of Mx Protox protein in both chloroplasts and mithochondria with 5- and 12-fold increase in Protox activity, respectively, over the wild-type rice (Jung *et al*., 2004).

Resistance Management

In terms of management, fitness costs may be manipulated in programs which judiciously rotate herbicide modes of action so that counterselection can reduce the frequency of resistant phenotypes in years when alternative herbicides or other control techniques are used (Pedersen *et al*., 2007). Some precautions must be taken into account for the preventative or management of resistance in weeds including herbicide rotation (different modes of action and different crop selectivities), crop rotation, rotation of weed control measures (mechanical weeding, bioherbicides, cover plants, the use of clean seeds) and lowering selection pressure. Lowering selection pressure by application of lower amounts of herbicides could facilitate the development of non-target resistance (Gressel and Rotteveel, 2000). They discussed the danger of creeping resistance with different minor mutations leading to a polygenic resistance or gene amplification especially when the dose is gradually increased. Their recommendation was to use a sequence of low doses followed by a moderate dose. The moderate dose should be sufficient to control individuals with low resistance. It is also of importance to recognize the mode of action and resistance in order to be able to choose an adequate management option and also to monitor resistance (Shaner, 1995). To delay the evolution of herbicide resistance in weeds, herbicides must be used in mixtures to stack two genes for herbicide resistance. It considerably lowers the mutation frequency for resistance in the weed (Powles *et al*., 1998; Cobb and Kirkwood, 2000). Simply combining herbicides with different modes of action will not result in delaying resistance if the efficacy and temporal activity characteristics of the mixed herbicides do not match. Both mixing partners must effectively inhibit the weeds most sensitive to the vulnerable herbicide (Maxwell *et al*., 1990). Resistance could quickly evolve in a weed species that is naturally resistant to one of the herbicides in a mixture. The ideal mixing partner should have three other properties in addition to equal persistence (Gressel, 2000). It should have a different target site of action from the vulnerable herbicide. The mixing partner should not be degraded in the same manner as the vulnerable herbicide. The mixing partner would be to possess negative cross-resistance. This would actually reduce the frequency of resistant alleles in the weed population (Gadamski *et al*., 2000).

Hazards of the new herbicide resistance technology have sometimes been compared to hazards of other currently used herbicides or alternative weed control measures (Gressel, 2000). Several environmental problems related to plant genetic engineering prevent realization of its full potential. One such common concern is the escape of foreign genes through pollen dispersal from transgenic crop plants engineered for herbicide resistance to their weedy relatives creating superweeds or causing gene pollination among other crops (Daniell, 1999). Such dispersal of pollen from transgenic plants to surrounding non-transgenic plants has been documented. Kling (1996) mentioned that herbicide resistant crops can lead to the evolution of 'superweeds' that will inherit the earth. Biotechnologically-derived herbicide-resistant crops can be of great benefit only if used with care to prevent or mitigate gene transfer to related feral and wild related weeds. The main risk is claimed to be that of the biotechnologically-derived herbicide resistant crops becoming 'volunteer' weeds (in following crops), or their introgressing traits into a wild relative rendering it weedier (Gressel and Rotteveel, 2000). Risk assessment must be performed on a local or regional basis, as the risks from the same herbicide resistant crops will vary greatly from one agricultural ecosystem to another. The final decision on risk/benefit is ultimately a balance between science, economics, local benefits, local values, as well as local politics (Powell, 1997).

There are some mechanisms that can be used to prevent or mitigate the risk of introgression (Gressel, 1999a; Gressel and Rotteveel, 2000). The particular placement of a transgene in crop genomes can affect its movement to other varieties and species. Only one of the genomes of the crop is identical to that of a related weed allowing easy gene transfer. For example, the D genome of wheat is compatible with the D genome of *Aegilops cylindrica* and the B genome of oilseed rape is also compatible to many brassica weeds (Zemetra *et al*., 1998). Consequently, transgenes easily introgress from wheat or oilseed rape to their relative wild species. Therefore, the molecular mechanisms if will not be entirely failsafe, at least they should dramatically reduce introgression risks to minimal levels.

CONCLUSION

The basis of herbicide selectivity is the ability of crop plants to survive the application of an herbicide at a specific rate while the weeds are injured or killed. Nevertheless, the frequent usage of herbicides can increase the number of herbicide-resistant weeds. Plants metabolize several herbicides to insoluble residues mainly through oxidative reactions catalyzed by P450s and/or conjugation with GSH by GSTs. P450-based metabolism gives widespread cross resistance in plants. The enhanced metabolism in P450 resistance mechanism is combined with other resistance mechanisms resulting in biotypes with multiple resistance mechanisms conferring resistance across many herbicide chemistries.

Concluding Remarks

The development of multiple resistance results from the accumulation of many resistance mechanisms. The most common forms of cross resistance to herbicides are conferred by target site cross resistance mechanisms. Control of target site-based resistant plants can be achieved by the use of herbicides with different modes of action. Uses of molecular biology are to find new herbicide targets and to generate herbicide-resistant crops. The insertion of exogenous resistance genes into the crops leads to transgenic herbicide resistant crops. Unlike the target site resistances, the crops generated with metabolic resistances seem to be problem-free. There is a fitness penalty with any target site herbicide resistant crops. Transgenic plants with target site resistance are functionally heterozygous. Stacked two genes for herbicide resistance by using herbicide mixtures should delay the resistance. Precautions must be considered for risk assessment. The escape of foreign genes through pollen dispersal from transgenic crops engineered for herbicide resistance to their weedy relatives creating superweeds or causing gene pollination among other crops must be taken into consideration. The main risk is claimed to be that of the biotechnologically-derived herbicide resistant crops becoming 'volunteer' weeds, or their introgressing traits into a wild relatives. The final decision on risk/benefit is ultimately a balance between science, economics, local benefits, local values, pressure groups, as well as local politics.

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